

STRUCTURE AND FUNCTION OF THE HEMISPERMATOPHORE AND SPERMATOPHORE OF *BOTHRIURUS FLAVIDUS* KRAEPELIN, 1910 (SCORPIONES, BOTHRIURIDAE)¹

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ABSTRACT

The morphology and functioning of the spermatophore of *Bothriurus flavidus* Kraepelin, 1910 are studied. Comparisons were made between 16 hemispermatophores, nine pre-insemination spermatophores and eight post-insemination spermatophores. The similarities and differences between the spermatophores of this and other species of genus *Bothriurus* Peters, 1861 are established. Previous studies on bothriurid spermatophores are discussed. The associated behaviours to process capsular ejection and the following expulsion of sperm into female are added.

KEYWORDS. Spermatophore, *Bothriurus flavidus*, sperm transfer, Argentina.

INTRODUCTION

One reason for a thorough examination of scorpion spermatophores is based upon the fact that this order probably represents an ancestral group in the evolution of the arachnids sperm transfer mechanisms (ALEXANDER, 1964; THOMAS & ZEH, 1984). The analysis of the different morphological and/or functional types of spermatophores, in each family, contributes to the knowledge of basic patterns, as well as to the specific features exclusive to each "type". ANGERMANN (1955, 1957) working on Chactidae, ALEXANDER (1957) on Scorpionidae, and ROSIN & SHULOV (1963) on Diplocentridae, among others, have established the bases for such studies on spermatophores of "lameliform" type (nomenclature after FRANCKE, 1979).

The spermatophores of the family Bothriuridae have scarcely been studied. Previous studies have focussed either on the morphology (SAN-MARTIN & GAMBARDELLA, 1967; MAURY, 1975) or on the mechanism by which they

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transfer sperm. MAURY (1968) studied the function of a spermatophore of *Urophonium iheringi* Pocock, 1893; ACOSTA (1989) and PERETTI (1992) of *Bothriurus bonariensis* (C.L. Koch, 1843), a species that had been partially analyzed by ZOLESSI (1956).

The spermatophore of *Bothriurus flavidus* Kraepelin, 1910 has been previously described by ABALOS & HOMINAL (1974), based on partially everted spermatophores. The objective is to describe the morphology of the hemispermatophore and spermatophore in *B. flavidus* as well as its functioning in order to provide a future comparative study of spermatophores of the family Bothriuridae.

MATERIAL AND METHODS

Sixteen hemispermatophores were studied, as well as nine preinsemination spermatophores and eight post-insemination ones, produced by 20 males of *B. flavidus*. The specimens were collected in December 1990 and 1991 near Sierra de la Ventana, province of Buenos Aires, Argentina.

The hemispermatophores were dissected from the paraxial organs, the membranous structures of the male genital system which contains them. Appropriately cleaned of all covering tissues, they were preserved in 80° ethyl alcohol. The spermatophores were removed from the substratum before or after the sperm transfer, and then stored with the specimens as the hemispermatophores, deposited in the Cátedra Diversidad Animal I, Universidad Nacional de Córdoba, Argentina.

The nomenclature proposed for the spermatophore of *B. bonariensis* by PERETTI (1992) is followed. When pointing out distal-proximal, dorsal-ventral and internal-external direction, the actual position of the organs in the male body is taken into account. A total of 18 mating behaviours was observed in December 1990, January and December 1991, and January 1992, in a 60x50x15cm arena. The substratum consisted of soil provided with 5-60mm, diameter stones and pieces of wood or tree barks. The observation was related and recorded in cassettes tapes. Many representative parts of the mating were filmed. For more details of this methodology see PERETTI (1991). The abbreviations used here deriving from the spanish names of the structures.

RESULTS

Hemispermatophore (figs. 1-9). Light chestnut colour, weakly sclerotized and very translucent. Lamina (LA) shorter than the trunk (TR), visibly inclined towards dorsal (figs. 1,2). Crest (CR) well developed with a transversal wall (PT) either near its proximal limit or rising from the base (fig. 6). Dorsal edge (FD) long, occupying the middle and distal part of the lamina. Brief dorsal fold (RD), poorly developed, enlarged towards distal end. Ventral fold (RV) is continued in the trunk, marking the boundaries of semicircular depression (DS) (fig. 4), and reaches to the disto-external region of the capsule (CA), finishing in an acute point.

The external and internal capsular invaginated sheets (A, B), of regular development, internally pointed slightly towards ventral (fig. 5). The capsular lobe (LC) wide, with slightly undulated borders, and somewhat concave at its ventral face (figs. 7,9). The distal end of this lobe parallels in position and length the extreme of the capsular sheet "A" (figs. 3,5). The capsular concavity (CC), slight, and placed nearly in the middle of the internal sheet (figs. 5,7). The trunk wide, but gradually narrowed towards the proximal end. Capsular flexure (FC)

mild, which delimits a flat truncal convexity (CT) (figs. 4, 8). Pedal flexure (FP) thin and pedicel (PE) oval.

Pre-insemination spermatochore (figs. 10-12). The spermatochore remarkably translucent, makes it possible to observe clearly the sperm stored in the trunk as this region acquires a yellowish-white tone. The dorsal edge of the lamina noticeably well developed (fig. 10).

The capsular lobes included in the capsular aperture (AC); only the distal tips can be seen from the outside. Both lobes remain attached by a thin layer of adhesive substance. These lobes (in relation to their orientation in the hemispermatochore) experimented a half torsion towards dorsal upon their external face, resulting in their concave surfaces being oriented to the external side.

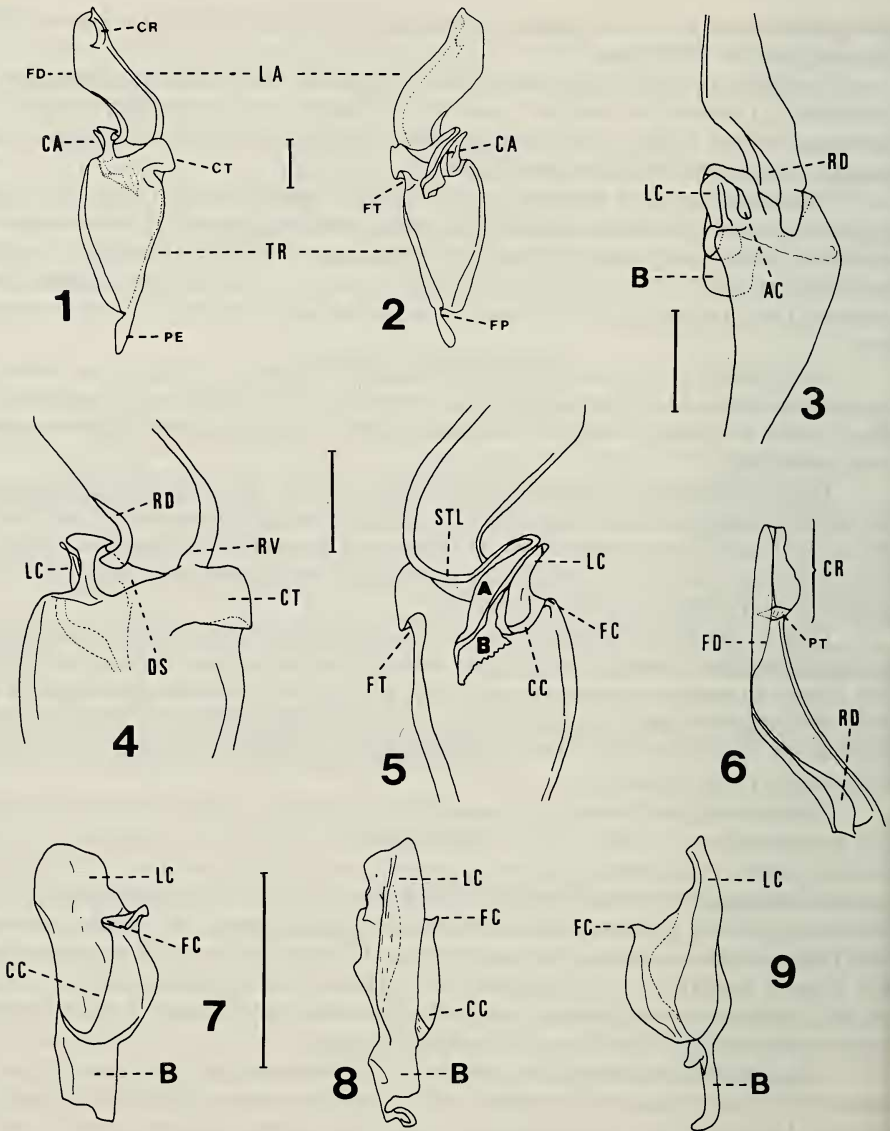
The trunk little stylized, somewhat amphora-shaped in dorsal view; wider at the truncal flexure (FT) level (fig. 10). The pedicel (PE) fixed to the substratum (fig. 11) by a cement, which is translucent when deposited and slightly amber when solidified.

Post-insemination spermatochore (figs. 13-16). The capsule, evaginated, like a semicollapsed cone (figs. 13,15), bears a rounded foramen (FO) at its tip (figs. 13, 15, 16). The capsular lobes (LC) remain generally held together (fig. 14), now oriented towards the proximal aspect and resting on the back of trunk, showing their slightly concave surfaces.

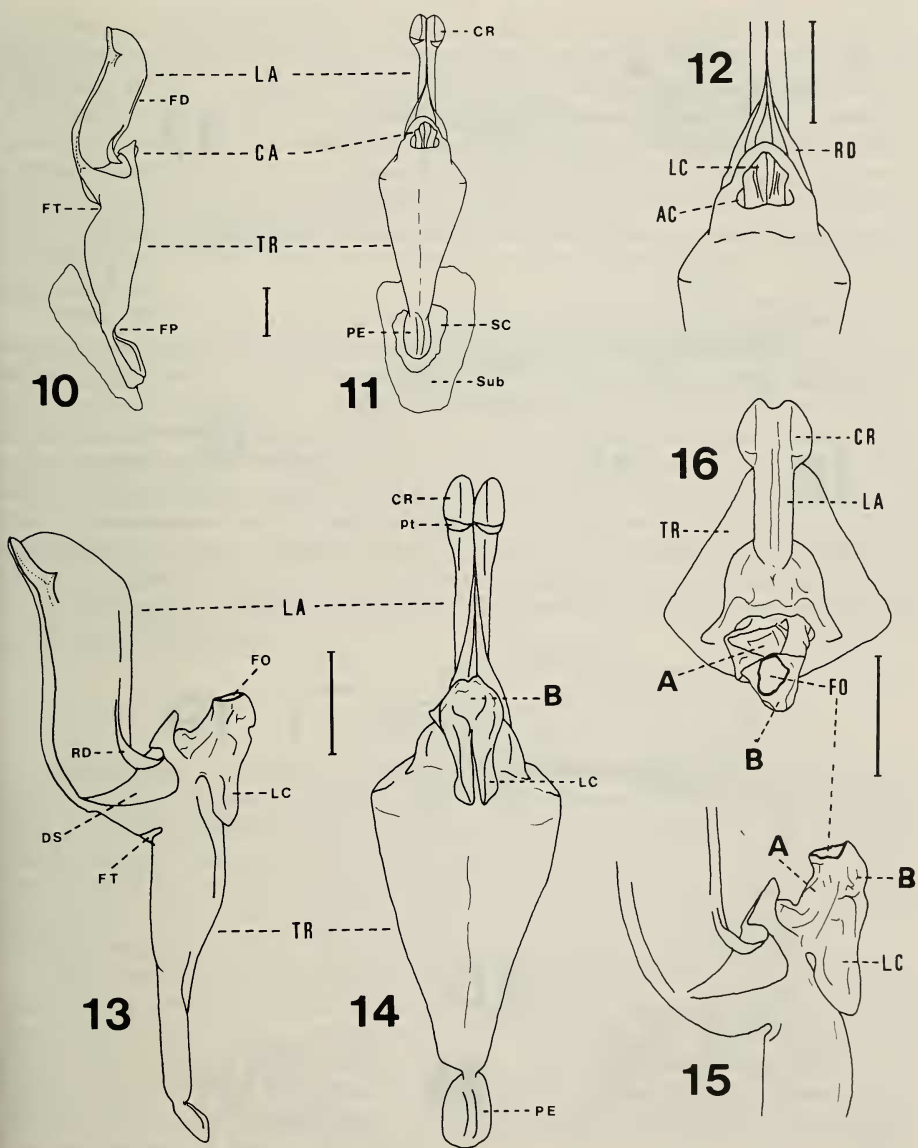
Due to the weak consistency of the spermatochore, the trunk is completely collapsed ventro-dorsally. This is particularly true at truncal flexure level (fig. 13), where it usually remains pressed by the lamina, either forming an angle of 45° or recovering a position similar to the original condition (figs. 13-16). After the process of sperm transfer, the spermatochore generally recovers its original orientation to the substrate.

Functioning mechanism and associated behaviours. Once the male deposited the spermatochore, what takes $1 \text{ min } 31\text{s} + 5.3\text{s}$ ($N=11$), the positioning of the female above the spermatochore is carried out. In this moment, she has her genital opening uncovered (fig. 17). During this behaviour the palpal chelae of the female were still grasped by the male, as it was during the whole previous courtship. At the same time the male grasped with its chelicera the region around the female mouth. The approximation becomes more slowly, till the dorsal surface of the spermatochore lamina fits between the female's coxae, and the capsular lobes lightly penetrate her genital opening.

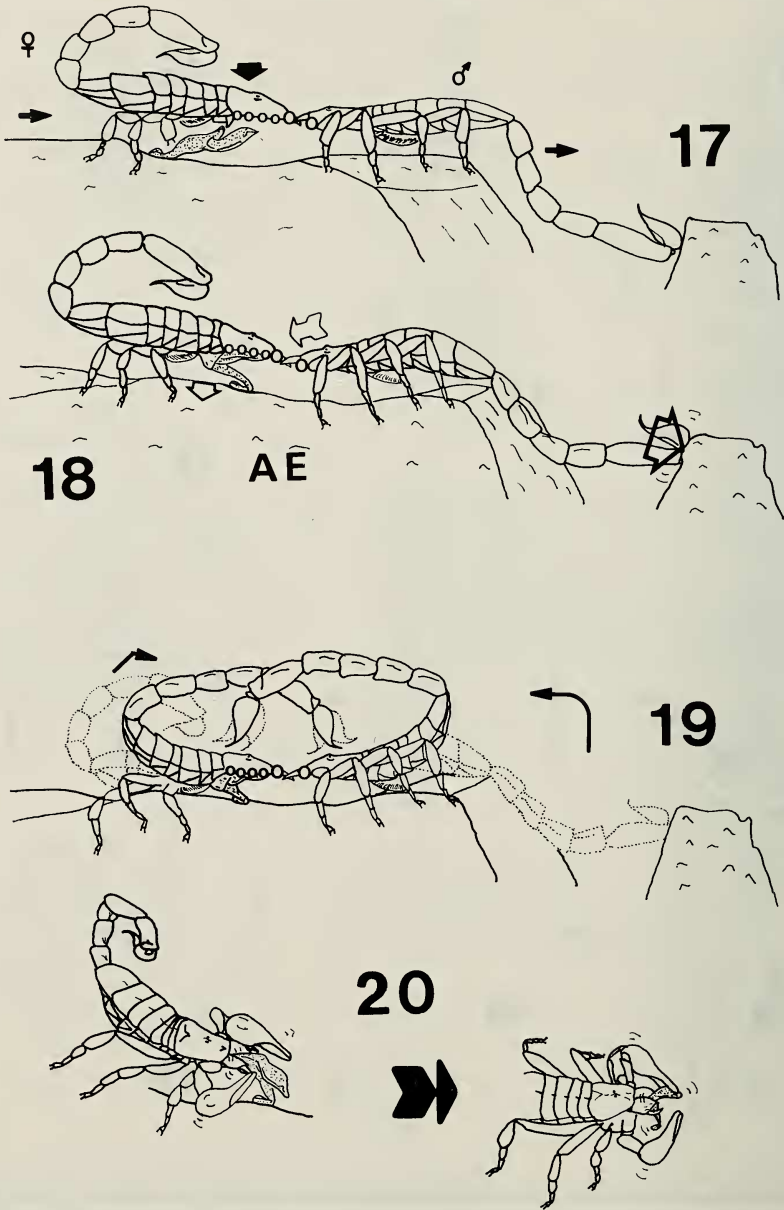
Then the male releases the chelae of its partner and holds her second pair of legs with his palps, near by the femur-tibia joint. Here starts the behavioural unit called "Action on the spermatochore" (AE) (fig. 18) which takes $1 \text{ min } 20\text{s} + 6.8\text{s}$ ($N=10$). This unit is guided and conducted by the male, which supports the extreme of his metasoma on the substratum (a stone for example). The male pushes the female backwards and downwards, causing in this way the bending of the spermatochore, while at the same time, part of the trunk and the lamina enter her genital opening. If this is successfully performed, the complete capsular eversion and the ejection of the total sperm contents, both occurring in the inner of the female genital tract, will be produced.



Figs. 1-9. *Bothriurus flavidus* Kraepelin, 1910; Left hemispermaphore: 1, external face. 2, internal face. Detail of the capsular region: 3, dorsal face; 4, external face; 5, internal face; 6, dorsal face of the lamina. Detail of the capsular lobe: 7, dorsal view; 8, ventro-internal view; 9, ventro-external view (in these drawings the capsular sheet B is not complete). Abbreviations: A, external capsular sheet; AC, capsular aperture; B, internal capsular sheet; CA, capsule; CC, capsular concavity; CR, crest; CT, truncal convexity; DS, semicircular depression; FC, capsular flexure; FD, dorsal edge; FP, pedal flexure; FT, truncal flexure; LA, lamina; LC, capsular lobe; PE, pedicel; PT, transversal wall of the crest; RD, dorsal fold; RV, ventral fold; STL, laminar-trunk suture; TR, trunk. Scale lines= 1mm.



Figs. 10-16. *Bothriurus flavidus* Kraepelin, 1910. Spermatochore; pre-insemination condition: 10, right view; 11, dorsal view; 12, dorsal view of capsular region. Post-insemination condition: 13, right view; 14, dorsal view; 15, dorsal view of capsular region; 16, distal view of capsular region. Abbreviations: A, external capsular sheet; AC, capsular aperture; B, internal capsular sheet; CA, capsule; CR, crest; DS, semicircular depression; FD, dorsal edge; FO, foramen; FP, pedal flexure; FT, truncal flexure; LA, lamina; LC, capsular lobe; PE, pedicel; PT, transversal wall of the crest; RD, dorsal fold; SC, cement-like substance; Sub, fragment of substratum; TR, trunk. Scale lines = 1mm.



Figs. 17-18. *Bothriurus flavidus* Kraepelin, 1910. Sequence of using of the spermatophore: 17, positioning of the female over the just deposited spermatophore by the male; 18, behavioural unit action on the spermatophore (AE); 19, attempt to mutual stinging after AE is finished (the female is still penetrated by the spermatophore); 20, taking and eating of the post-insemination spermatophore by the female.

During AE unit the body of the male shows a series of curvatures (fig. 18), just like when the spermatothore is deposited: mesosoma convex to dorsal and metasoma convex to ventral. This is due to the pressure performed by the male. The female lays more downwards and backwards on the spermatothore, without showing its body significative curvatures. Once the flexion of the spermatothore reaches a maximum and the sperm transfer is finished, the body of the male relaxes. In this way he stops to push his partner, and at the same time the cited flexion of the spermatothore diminishes. At this point the unit is concluded, although the female keeps on being penetrated a few seconds by the empty spermatothore.

After AE, one of the partners, generally the male, try to sting the other, causing it to respond in the same way (fig. 19). The male releases the female and moves quickly away while the female normally - 73% (8/11) - remains in the same place, and eats the post-insemination spermatothore (fig. 20). The female exhibits sometimes an early attempt to sting, either while offering resistance to be placed on the spermatothore - 16% (2/12) - or before AE is finished - 33% (4/12). This causes that the male releases the female, leaving the sequence incomplete. In other circumstances the AE unit is partially or defectively performed, without producing an adequate capsular evertion of the spermatothore, with little (10-30%) or no ejection of sperm. Very often those difficulties arise when performing AE on substratum which for example do not provide suitable supporting points for the male's metasoma.

DISCUSSION

The general morphology of hemispermatothores and pre-and post-insemination spermatothores of *B. flavidus* show close similarities to the structural type which is also observable in other species of the same genus (Acosta, personal communication), such as *B. conspicuus* Mello-Leitão, 1934, *B. burmeisteri* Kraepelin, 1834, *B. bucherli* San Martín, 1963, *B. voyati* Maury, 1973, and *B. rochensis* San Martín, 1965. These species exhibit, however, clear differences in their external morphology (MAURY, 1973; Acosta, personal communication).

The formerly mentioned species shares lamina with long dorsal edge, marked crest, slightly developed dorsal fold, and spatula-like capsular lobes, which hardly appear visible outside the capsular aperture. These are general similarities, but several differences of minor magnitude also exist, for example, the size in the shape of the lobes and the presence or absence of spines and apophyses in the distal-external border of the capsule. These differences could have some relation to a mechanism of interspecific reproductive isolation at the level of sperm transfer (PERETTI, 1992).

The spermatothores in this group of species are easily distinguishable from other forms of spermatothores in the genus *Bothriurus*, such as *B. bonariensis*, recently studied by ACOSTA (1989) and PERETTI (1992). Compared with the spermatothore of *B. flavidus* and allied species, that of *B. bonariensis* has a lamina with a short dorsal edge, small crests and well developed dorsal fold and

capsular lobes. According to this, the spermatophore of *B. flavidus* and related species constitute one of the most prominent "fundamental models" within *Bothriurus* (MAURY, 1980).

ABALOS & HOMINAL (1974) studied specimens of *B. flavidus* from the "Pampa de Achala" province of Córdoba, Argentina. Although slight differences between the populations occur in spermatophore morphology as exomorphology (MAURY, 1973; ACOSTA, 1989), it is evident that the authors described the spermatophore in the pre-insemination condition, although its capsule was indeed partially evaginated. This fact was first noticed by ACOSTA (1989), who correctly illustrates an pre-insemination spermatophore.

It might be pointed out that the functioning mechanism of the spermatophore of *B. flavidus* follows the same pattern of *B. bonariensis*, which has been analyzed in detail (PERETTI, 1992), and the rest of the family Bothriuridae. However, the AE unit takes longer in *B. bonariensis* (5-6 min).

According to the observed behavioural and morphological features, it is deduced that in Bothriuridae the capsular eversion occurs because of the pressure performed by the female body on the lamina, when pushed by the male. This bending of the spermatophore at the trunk flexion determines an internal pressure of the sperm content, causing the whole invaginated structures to unfold through the capsular opening, and the sperm to ooze through the newly formed foramen. This aspect was analyzed by PERETTI (1992), confirming that the pressure on the lamina in order to cause the eversion of the capsule should be conducted towards proximal and ventral, that is a bidirectional force, with a more important horizontal component; one of the two directions alone is not enough.

The difference between AE unit duration of *B. flavidus* and *B. bonariensis* may be due to the distinct consistences of the respective spermatophores, as the ones of *B. bonariensis* show a strongly sclerotization. This affects noticeably the time needed to pressure on the lamina, as to get the whole evagination of the whole capsule and the complete ejection of the sperm. Although the capsular region in Bothriuridae shows greater capsular complexity than in the rest of the families with "lameliform" spermatophore (MAURY, 1980), and its way of eversion seems more elaborated too, the ethological pattern of AE could be basically similar (FRANCKE, 1979; POLIS & SISSOM, 1990; BENTON, 1993) in the sense that all of them might show (ANGERMANN, 1955, 1957; ALEXANDER, 1957) a functioning of "lever" type.

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